3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-Histone H2AX [pSer^{139}]
Developed in Rabbit
IgG Fraction of Antiserum

Product Number **H 5912**

Product Description

Anti-phospho-Histone H2AX [pSer^{139}] is developed in rabbit using a synthetic, phosphorylated peptide corresponding to amino acids 134-142 [pSer^{139}] of human histone H2AX C-terminus, conjugated to keyhole limpet hemocyanin (KLH) as immunogen. This sequence is highly conserved (single amino acid substitution) in mouse histone H2AX. Whole antiserum is fractionated and then further purified by ion-exchange chromatography. The resulting IgG fraction is further purified by absorption on the non-phosphorylated histone H2AX peptide (human, amino acids 134-142).

Anti-phospho-Histone H2AX [pSer^{139}] recognizes histone H2AX phosphorylated on Ser^{139} (γ-H2AX) by immunoblotting (15 kDa). Staining of [pSer^{139}] phosphorylated histone H2AX in immunoblotting is specifically inhibited with the [pSer^{139}] phosphorylated histone H2AX immunizing peptide (human, amino acids 134-142). No inhibition is observed with the respective non-phosphorylated histone H2AX peptide (human, amino acids 134-142).

Histone proteins (H3, H4, H2B, and H2A) function as building blocks to package eukaryotic DNA into repeating nucleosome units that are folded in higher-order chromatin fibers.[1] The nucleosome is composed of an octamer containing an H3/H4 tetramer and two H2A/H2B dimers, surrounded by approximately 146 base pairs of DNA. The H2A subfamilies include H2A1-H2A2, H2AZ, and H2AX.[2] H2AX comprises 2-10% of the total H2A complement in mammalian cells, whereas in the budding yeast it constitutes virtually all of the H2A molecules. H2AX becomes rapidly phosphorylated on Ser^{139} (known as γ-H2AX) at a very early response of mammalian cells to DNA double-strand breaks (DSBs), generated by ionizing radiation, endogenous oxygen reactive species, during apoptosis, during meiosis at sites of recombinational DSB, and at sites of V(D)J recombination in developing thymocytes.[3-7] DSB result in discrete γ-H2AX nuclear foci at the DNA damage sites, cause chromatin decondensation, and are required for efficient DNA DSB repair. In addition the phosphorylation of H2AX appears to play a critical role in the recruitment of repair factors to the site of DNA damage. H2AX phosphorylation at Ser^{139} occurs in the unique C-terminal motif KKATQASQEY that differentiates H2AX from the other H2As. Members of the PI3-kinase family including DNA-PK, ATM, and ATR are involved in the responses of mammalian cells to DSBs, and have all been implicated in H2AX phosphorylation.[8, 9]

ATM is the major kinase involved in the phosphorylation of H2AX in response to DNA double-strand breaks.[9] H2AX knockout mice are radiation sensitive, growth retarded, immune deficient, and mutant males are infertile.[10] These pleiotropic phenotypes are associated with chromosomal instability, repair defects, and impaired recruitment of repair factors to irradiation-induced foci, indicating that H2AX is critical for facilitating the assembly of specific DNA-repair complexes on damaged DNA.

Reagent

Anti-phospho-Histone H2AX [pSer^{139}] is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

For immunoblotting, a minimum working antibody dilution of 1:1,000 is recommended using a whole cell

extract of the HeLa (human epitheloid carcinoma) cell line or the NIH3T3 mouse fibroblast cell line treated with staurosporine.
Note: In order to obtain the best results using various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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ER/KAA 02/03